AUG -9 1948

VOLUME 6 ©CIB 148240 NUMBER 8

NUTRITION REVIEWS

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Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

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NUTRITION REVIEWS

VOL. 6 AUGUST, 1948 NO. 8

HISTORICAL ASPECTS OF PROTEIN NUTRITION

A conception of the chemical nature of proteins was not possible until their hydrolysis products were identified. The products of alkaline hydrolysis undergo extensive decomposition and accordingly it was through the study of the products of acid hydrolysis that it became evident that hydrolytic cleavage yields alpha-amino acids. About eighty years elapsed between the first separation of amino acids from hydrolysates and the accumulation of sufficient data to indicate that only alpha-amino acids in peptide linkage form the simple proteins.

H. Braconnot (*Ann. Chim. phys.* (*Series 2*) **13,** *113* (*1820*)) discovered glycine and leucine; F. Bopp (*Liebig's Ann. d. Chemie u. Pharm.* **69,** *16* (*1849*)) discovered tyrosine; E. Cramer (*J. prakt. Chemie* **96,** *76* (*1865*)) discovered serine; H. Ritthausen (*Ibid.* **99,** *454* (*1866*)) discovered glutamic acid and (*Ibid.* **106,** *445* (*1869*)) aspartic acid; E. Schulze and J. Barbieri (*Ber. d. deutsch. chem. Gesellsch.* **14,** *1785* (*1881*)) discovered phenylalanine. Thus, in 1881 seven amino acids had been isolated and identified among the acid hydrolysates of proteins. Already the idea had occurred that amino acid deficiency might determine protein inadequacy.

Some of the characteristic properties of proteins had been familiar to observing people from time before history in such substances as curds of milk, white of egg, and gelatin in water in which meat had been boiled. F. Magendie ("Précis élémentaire de physiologie," *Paris* (*1816*)) appears to have been the first to perceive that there exist several kinds of nutrient principles, among them proteins. The term protein was coined by G. J. Mulder (*J. prakt. Chemie* **44,** *503* (*1848*)). Magendie restricted a dog to a diet of gelatin and observed that it failed nutritionally.

The similarity of gelatin to protein aroused speculation concerning its nutritive value. C. Voit (*Z. f. Biol.* **8,** *297* (*1872*); **10,** *202* (*1874*)), discussed the problem. He showed by experiment that when a dog was given gelatin only, or gelatin with fat, the animal excreted more nitrogen than it ingested in its diet. When, however, a small increment of protein was added to the gelatin, nitrogen equilibrium was brought about. Voit said that the gelatin spared protein of the tissues by preventing its metabolic dissolution. But he added that such sparing of protein can be accomplished also by fat and by carbohydrate although not to the same extent as by gelatin.

F. Hofmeister (*Z. f. physiol. Chem.* **2,** *299* (*1878*)) published analyses of gelatin from several sources. He was familiar with the fact that gelatin does not give the Millon reaction (a test for tyrosine). The first attempt to investigate the possibility that gelatin supplemented with tyrosine could replace protein in nutrition was made by L. Hermann and T. Escher (*Vrtljschr. d. Naturf. Gesellsch. in Zürich, p. 36* (*1876*)). Their results seemed to answer the question in the affirmative, but further experiments gave negative results.

K. B. Lehmann (*Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. in München* (*1885*)) fed 2 rats a mixture of gelatin, rice starch, a butter-margarine mixture, meat extract, and bone ash supplemented with tyrosine. The rats died on the forty-seventh and seventieth day, respectively. The conclusion was drawn that gelatin plus tyrosine was not the equivalent nutritionally of protein.

The hypothesis of Voit and Pflüger that the protein molecule undergoes metabolism as a whole was not satisfying to many physiologists and there was a steady but

slow advance of knowledge through experiments designed to reveal the significance of the amino acids rather than "protein" in nutrition. As a basis for such studies gelatin long remained of special interest. In 1899, K. A. H. Mörner (*Öfversigt kgl. Vetenskaps-Akad. Förh.* (*Swedish Academy*), *p. 167* (*1899*)) announced the discovery of cystine in hydrolysate from horn. He showed that cystine is lacking in gelatin. F. G. Hopkins and S. W. Cole (*Proc. Roy. Soc.* (*London*) **68,** *21* (*1901*)) announced the identification of tryptophan, the substance which had hitherto been known among protein cleavage products which give the Adamkiewicz reaction (A. Adamkiewicz, *Pflüger's Arch. f. d. ges. Physiol.* **9,** *156* (*1874*)). This amino acid proved also to be lacking in gelatin. M. Kauffmann (*Ibid.* **109,** *440* (*1905*)) was the first to test by experiment whether gelatin supplemented with tyrosine, cystine, and tryptophan as the sole source of "protein" in the diet of a dog would prove adequate. Both his experiment with dogs and a subsequent one on himself showed that gelatin thus supplemented was capable of covering the protein requirement of the body.

The events in the history of the study of the chemical composition of proteins cannot be presented here. Ritthausen, Hoppe-Seyler, Weyl, Schmiedeberg, Drechsel, and others contributed bits of knowledge concerning the usefulness of dilute solutions of neutral salts as solvents for proteins, and, in greater concentrations as precipitants for them. Then followed the notable investigations of Kuehne, Hofmeister, Kossel, E. Fischer, Osborne, Abderhalden, Van Slyke, Dakin, and Vickery, whose studies established the behavior of proteins toward reagents of various kinds, the polypeptide nature of the protein molecule, the nature and extent of protein cleavage by enzymes, the yields of different amino acids to be secured from a considerable number of proteins, and the extension of the discovery of amino acids until no less than twenty-three have been shown to arise from acid hydrolysis of proteins. Van Slyke devised analytical procedures which enabled him to determine the extent of protein digestion as hydrolysis proceeded. He was able to follow amino acids arising through digestion in the alimentary tract during absorption, distribution by the blood stream to the tissues, and their abstraction by the tissues which they were destined to nourish.

One of the first to study enzyme digestion of proteins was L. N. Vauquelin (*Ann. chim.* **43,** *267* (*1803*)) who studied the action of papayotin. C. Bernard (Leçons d. Phys. Exp., *p. 334, Paris* (*1855*)) discovered the proteolytic action of pancreatic juice, the enzyme of which was named trypsin by W. Kuehne and which he studied extensively. T. Schwann (*Poggendorf's Ann.* (*Series 2*) **8,** *358* (*1836*)) discovered pepsin. O. Cohnheim (*Biochem. Centralbl.* **1,** *169* (*1903*)) discovered erepsin in intestinal juice, and showed that it could digest peptides to the amino acid stage. This demonstration, together with the achievement of Van Slyke in tracing amino acids from the intestine to the tissues where they serve for resynthesis of proteins of specific constitution, shifted emphasis from the term protein to the term amino acids in later thoughts on protein nutrition.

W. Hausmann (*Z. f. physiol. Chem.* **29,** *136* (*1900*)) hydrolyzed protein with acid and determined in the hydrolysate the amount of nitrogen present as "humin," as ammonia, as basic amino acids (precipitable by phosphotungstic acid), and by difference, the mono amino acids in the sample. His results showed striking differences in the composition of the three proteins which he studied. Many of the proteins which had been isolated by T. B. Osborne were subjected to the procedure of Hausmann and, later to that of Kossel and Kutscher (Osborne and I. F. Harris, *J. Am. Chem. Soc.* **25,** *323* (*1903*); Osborne and R. D. Gilbert, *Am. J. Physiol.* **15,** *333* (*1906*); Osborne, C. S. Leavenworth, and C. A. Brautlecht, *Ibid.*

23, *180 (1908)*). The results of analysis of different proteins by this method were so strikingly contrasting that it appeared certain that proteins must differ markedly in their nutritive values depending on their quantitative yields of amino acids.

The earlier efforts to compare the nutritive values of isolated proteins from different sources by feeding experiments were unsuccessful because of lack of knowledge of the existence of vitamins and the inadequacy of the salt mixtures employed to provide the necessary inorganic elements. The earliest notable success in this field of inquiry attended the studies of T. B. Osborne and L. B. Mendel (*Publications of the Carnegie Institution of Washington, Bull.* **156** (*Parts I and II*) (*1911*)). They abandoned efforts to feed purified protein, carbohydrate, fat, and salt mixtures after unsuccessful trials, and devised a basal diet which overcame almost all difficulties which had hitherto stood in the way of success in such experiments. They employed as a prominent constituent of the basal diet "protein-free milk." This was prepared by acidifying to precipitate casein, and subsequent boiling to coagulate lactalbumin. These proteins were removed by filtration and the resulting whey was evaporated to dryness. It was a yellow solid composed principally of lactose but containing the ash constituents of milk except the phosphorus removed in the casein. It provided all of the water soluble vitamins and some carotene which served as a precursor of vitamin A. Its one shortcoming was that it contained a considerable amount of nitrogen in simple forms, some of which was amino acids. Using a basal diet containing 28.3 per cent of this preparation they were able to make diets containing a single isolated protein which were complete as sources of nutrients. The success or failure of the growth or maintenance experiment, using young rats as subjects, turned on the quality of the protein as a source of amino acids. The presence of some amino acids of unknown identity in the "protein-free milk" prevented the comparisons of nutritive values of a series of purified proteins from being strictly quantitative, nevertheless, the indispensability of lysine and tryptophan was demonstrated convincingly. Their studies were of outstanding importance in that they established that different proteins have widely contrasting values as sources of the essential amino acids for animal nutrition.

One of the first successful experiments which demonstrated that a mixture of amino acids could replace protein in the diet was reported by E. Abderhalden (*Z. f. physiol. Chem.* **78,** *1 (1912)*). He restricted a dog during one hundred days to a diet in which the sole source of amino acids was a digest of meat which had been successively subjected to hydrolysis by pepsin, trypsin, and erepsin, and was shown to have been digested to the amino acid stage. This digest was supplemented with non-nitrogenous nutrients. The dog remained in good health and gained 9.35 kg. in body weight. The animal must have synthesized from the amino acids ingested a large amount of protein for growth.

The development of ideas which matured in an understanding of the fact that with the exception of glycine, which contains no asymmetric carbon atom, amino acids derived from proteins possess optical activity, and that but one of the enantiomorphs of the synthetic, optically neutral mixture possesses biologic value (with certain exceptions), began with the observation of J. B. Biot (1774–1862) who observed that there occur in nature quartz crystals which have the property of rotating a ray of polarized light to the right and others which rotate to the left, when the ray is passed through the crystal in the direction of the long axis. He did not examine his crystals minutely or he might have discovered that the two varieties of quartz crystals possess structural peculiarities.

R. J. Haüy (1743–1822) one of the

founders of crystallography noted that there are in nature right- and left-handedness in quartz crystals in respect to the position of their hemihedral faces. He did not correlate the structural peculiarity of such crystals with the phenomenon of Biot. It was left for John Herschel (1792–1871) to test the rotatory power of Haüy's structurally right- and left-handed crystals on polarized light. He found that the mirror image crystal structure was associated with the direction of rotation of polarized light.

Biot observed that sugar, tartaric acid, and some other natural products are optically active in solution. Pasteur (1822–1895) in his study of the tartrates observed that crystals showing asymmetry in respect to hemihedral facets occur, and that these, when tested in solution showed opposite rotatory power. Paratartrates (racemic form), however, showed symmetry in crystalline structure (two epaulets) and possess no rotatory power. Pasteur next discovered that, when Penicillium mold is allowed to grow upon a solution of ammonium paratartrate, the right-handed (right-rotating) form decomposed and the left-rotating form remained in the solution and could be recovered by crystallization. A living organism had selected a single optical form for nutriment but could not utilize the mirror image molecule.

The selective metabolic utilization of L-amino acids by molds was studied by E. Schulze and A. Likiernik (*Z. f. physiol. Chem.* **17,** *513* (*1893*)), but similar experiments were not tried on mammals until J. Wohlgemuth (*Ber. d. deutsch. chem. Gesellsch.* **38,** *2064* (*1905*)) showed that rabbits given racemic tyrosine, leucine, aspartic acid, and glutamic acid excrete the D-form in the urine.

The foregoing account of the development of ideas in the history of the studies of protein nutrition includes many of the most important discoveries to the year 1920.

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CHOLINESTERASE ACTIVITY IN NORMAL AND ANEMIC SUBJECTS

Although Sir Henry Dale in 1914 suggested that an esterase exists in the blood which would inactivate acetylcholine, and O. Loewi and E. Navratil (*Pflügers Arch. f. d. ges. Physiol.* **214,** *678* (*1926*)) demonstrated the existence of such a substance in 1926, the host of papers which have appeared since then have given little attention to the content of the enzyme in red cells. F. Plattner and O. Galehr (*Ibid.* **219,** *609* (*1928*)), using a biologic assay method, found that the cholinesterase activity of erythrocytes is greater than that of serum. J. C. Sabine (*J. Clin. Invest.* **19,** *833* (*1940*)) showed that the cholinesterase activity of erythrocytes is greater than that of plasma in both normal and pathologic subjects. A. Sawitsky, H. M. Fitch, and L. M. Meyer (*J. Lab. Clin. Med.* **33,** *203* (*1948*)) have found the cholinesterase activity of 1 ml. of red blood cells to range from 7.25 to 10.34 units with a mean value of 8.76. The mean corpuscular esterase activity varied from 6.19 to 8.69 units ($\times 10^{-10}$) with a mean value of 7.55 units ($\times 10^{-10}$). The esterase activity of a similar quantity of plasma was 1.50 to 5.00 units with a mean of 3.19 units. Although the activity varied markedly from individual to individual, within the same subject it remained rather constant.

Sabine (*loc. cit.*) found that in 3 patients with pernicious anemia in relapse the whole blood, red cell, and plasma esterase activities were low initially. During treatment the cell esterase returned to normal levels in four to nine days. This was followed by a further increase in activity and then a decline to normal levels in about six weeks.

The plasma esterase responded much more slowly, rising to normal in about six weeks. A general lowering of serum esterase in patients with secondary anemias and acute hemolytic anemia was reported by W. Antopol, A. Schifrin, and L. Tuchman (*Proc. Soc. Exp. Biol. Med.* **38,** *363* (*1938*)), while G. E. Hall and C. C. Lucas (*J. Pharmacol. Exp. Therap.* **59,** *34* (*1937*)) found no correlation between the serum esterase activity and the hemoglobin concentration and red and white cell counts.

A series of articles by J. E. Davis (*Am. J. Physiol.* **142,** *402* (*1944*); (see also *Nutrition Reviews* **5,** *188* (*1947*)) concerning the experimental production of a "hyperchromic" anemia in dogs which responds to antipernicious anemia therapy has aroused new interest in this subject. The anemia is described as being produced by choline and acetylcholine (*Am. J. Physiol.* **147,** *404* (*1946*)) and is reported as responding to treatment with antipernicious anemia substance. Concomitant with the production of the anemias, the cholinesterase activity of the dog serums fell to a low value. With the institution of antipernicious anemia therapy the red cell count, hemoglobin, and esterase activity of the serum returned to normal levels. Folic acid was reported as having the same effect as liver extract (*Science* **104,** *605* (*1946*)). Davis has also reported that folic acid administered to normal human subjects increased the serum esterase activity significantly within five hours. He also described an increase in an acetylcholine-like substance in the blood of patients with pernicious anemia which returned to normal levels after antipernicious anemia therapy.

In view of these reports, L. M. Meyer, A. Sawitsky, N. D. Ritz, and H. M. Fitch (*J. Lab. Clin. Med.* **33,** *189* (*1948*)) measured the cholinesterase activity of whole blood, red cells, and plasma in patients with pernicious anemia in relapse and followed the changes associated with therapy. They found that during relapse these values were below normal levels. Thus, the mean esterase activity of the red blood cells was 5.90 units ($\times 10^{-10}$) in 10 patients while in 4 patients the value was within the normal range. The plasma levels were low with an average activity of 1.60 units. Three to nine days after beginning effective antipernicious anemia therapy there was a rapid rise in red blood cell esterase. The red cell activity rose to above normal values, remained high for thirty-five to eighty-five days and declined to the average range. The plasma esterase activity remained unchanged at the initial low levels and did not rise until eighteen to thirty days after the onset of effective therapy. The slope of the plasma rise tended to parallel the slope of the erythrocyte rise as both returned to normal.

In 4 patients the initial rapid red cell cholinesterase rise occurred at the same time that a reticulocytosis was noted. In 2 cases, however, the reticulocytosis preceded the red cell esterase rise while in one case the reticulocytosis continued even when the red cell esterase activity was falling. Furthermore, when doses of folic acid were given which were suboptimal and which, consequently, were associated with only small reticulocyte peaks, a pronounced rise in red cell esterase occurred nevertheless. Thus the rise in red cell activity was probably not related to reticulocytosis. Again, the cholinesterase activity of the red cells did not seem to be related to the mean cell volume or to the increase in the number of red cells although in some instances some correlation seemed to occur.

From these observations it would seem that patients with pernicious anemia in relapse have erythrocytes and plasma that are abnormally low in cholinesterase activity. In other anemias, according to unpublished observations by Meyer and his associates, pernicious anemia alone shows consistently low cholinesterase activity. This would suggest that the lowered cholinesterase activity seen in pernicious anemia may be related to the metabolic disorder which is the funda-

mental disturbance in this disease. What this may be is a matter for speculation at the present time. Sabine (*loc. cit.*) postulated a specific defect in pernicious anemia with an inability to put out cholinesterase in adequate amounts in such cells as the marrow produces. Davis, in the papers cited, suggested that relapse in pernicious anemia is related to the increased activity of acetylcholine. He believes that this increased concentration produces a dilatation of the vascular network of the bone marrow with an increase in marrow oxygen tension. With this increase in oxygen tension an inhibition of the maturation of cells of the erythroid series occurs.

INTERRELATIONS OF CHOLINE AND METHIONINE IN CHICK GROWTH

Choline and methionine appear to have at least two roles in metabolism: (1) as essential tissue components, and (2) as methyl group donors. Some evidence (D. J. Mulford and W. H. Griffith, *J. Nutrition* **23,** *91* (*1942*)) indicates that these roles may be mutually exclusive although more data are needed to establish this conclusively.

D. S. McKittrick (*Arch. Biochem.* **15,** *133* (*1947*)) has studied the interrelations of choline, methionine, and betaine in chicks during the fourth week of life. The experimental approach has been discussed (*Nutrition Reviews* **6,** *169* (*1948*)). The birds used were single comb White Leghorns. For the first three weeks of life they received a commercial mash and during their fourth week the test diet was administered. The basal experimental diet was low in choline and contained isolated soybean protein or arachin as the protein source and thus was also low in methionine. In the supplemented diets l-cystine was supplied at a level of 0.55 per cent, choline at levels varying from 0.14 to 1.5 per cent. By simultaneous variation of the choline and methionine levels it was possible to determine the limit beyond which the choline content of the diet could not be decreased without reducing growth below optimum levels, regardless of the level of methionine. This level, termed the essential choline, was approximately 0.1 per cent of the diet. In a similar fashion the limit beyond which the methionine content could not be reduced, regardless of the level of choline, was determined. This essential level of methionine was 0.5 per cent of the diet. These essential increments of choline and methionine were found not to replace one another. For optimum growth the essential levels of choline and methionine must be supplemented with a replaceable fraction of choline or methionine or an equivalent mixture, e.g., 0.25 per cent methionine plus 0.45 per cent choline. It was found that betaine could replace the replaceable fractions of choline and methionine but not the essential quantities of either of these compounds.

Excess methionine depressed the rate of chick growth from a high of 6.18 per cent per day (0.94 per cent choline plus 0.5 per cent methionine in the diet) to a low of 3.35 per cent per day (0.94 per cent choline plus 1.28 per cent methionine in the diet). This growth retarding effect of methionine could be counteracted by addition of glycocyamine to the diet. It was suggested that glycocyamine exerted its protective action by virtue of its role as methyl acceptor. Such a transfer of methyl from methionine to glycocyamine could result, according to present concepts, in the formation of homocysteine. High dietary homocysteine, in separate experiments, had no growth depressing effect unless additional choline was added. Added serine also counteracted the growth retarding effects of excess methionine. Choline chloride at levels up

to 1.6 per cent at the essential level of methionine did not depress growth. These effects of methionine agree fairly well with the predicted behavior of a compound which can form both inactive and active enzyme-substrate complexes.

A straight line relation between the logarithm of the food intake and growth was observed whether growth was stimulated or depressed by the ration fed.

This work illustrates some of the complexities of the interrelations which exist between nutrients in certain species. It also presents an illuminating application of graphic methods hitherto unusual in the field of nutrition.

PARENTERAL NUTRITION WITH FAT EMULSIONS

Complete parenteral nutrition is hampered because suitable fat emulsions for intravenous alimentation have not been available. Suitable fat emulsions would afford a high caloric intake in a minimum of fluid volume, an important advantage in certain cases. With a sufficiently high caloric intake, the utilization of protein from parenteral sources would be maximal and the destruction of body protein as a result of a caloric deficit would be minimal.

The studies of J. M. McKibbin and associates have been discussed previously (*Nutrition Reviews* **3,** *302* (*1945*)). In these and in later investigations (J. M. McKibbin, R. M. Ferry, and F. J. Stare, *J. Clin. Invest.* **25,** *679* (*1946*)) it was shown that emulsions given intravenously in the dog cause gain in weight, change a negative to a positive nitrogen balance, and produce an increase in fat as measured by total carcass analysis. In these studies a 15 per cent fat emulsion was infused into dogs at a slow rate. Granulomatous lesions were found in the lungs and spleen, and to a less extent in the liver, after daily infusion for thirty to ninety days. The fat emulsion contained, in addition to fat, a soybean phosphatide preparation as a stabilizing agent. The question was raised whether this material might not be primarily responsible for the histopathologic lesions.

A study by H. S. Collins and other workers from the same laboratory (*J. Lab. Clin. Med.* **33,** *143* (*1948*)) describes experiments carried out to determine the tolerance of dogs to emulsions of 30 per cent fat and to the rapid administration of such emulsions, and to measure the length of time infused fat remains in the blood. An attempt was made also to determine whether the fat or the stabilizer is primarily responsible for the granulomatous lesions in the tissues.

It was interesting to find that fat could be given in an emulsion at least twice as concentrated as previously used and at a much more rapid rate. Thus the goal of administering a large number of calories in a limited fluid volume and in a relatively short time seems to be feasible. The 30 per cent fat emulsion used furnished approximately 3 calories per milliliter. Except for the first two or three days of the infusion period when there was considerable vomiting, the dogs tolerated the emulsion well. Even this, generally, could be controlled if the emulsion was given slowly, especially during the first half of the infusion period. An epidemic of distemper in the experimental colony proved to be of some help in that it furnished an opportunity to observe the effect of fat emulsions in the presence of infection. The first of the fat infusions in one animal was begun a few days after the dog became distemperous and for a time the infused fat constituted the sole source of calories. During this period the dog lost weight rapidly but probably less than would have been experienced had it not been receiving fat. During the second infusion period, identical to the first except that the dog had recovered from distemper,

the fat was well tolerated, weight gain was rapid, and the dog was in excellent condition at the end of the experiment.

Two animals were given infusions of a 15 per cent fat emulsion, 2 others a 2 per cent fat emulsion, and a final pair received an emulsion containing no added fat but simply the phosphatide preparation used as the stabilizing agent. Histopathologic changes were essentially the same in all the animals but were more accentuated in the 2 dogs receiving the phosphatide alone. Thus it would seem that the stabilizer was the cause of the granulomatous lesions.

There was a consistent decline in the hemoglobin and volume of packed red cells during the course of the infusions. The degree of change did not seem to be dependent on the amount of fat in the emulsion. Since there was no significant reticulocytosis it would not seem that the anemia was due to blood destruction. Judged by excretion of bromsulfalein and by plasma total cholesterol and cholesterol ester levels, there was little evidence of impairment of liver function although this may have been a small factor. Plasma phosphatase values showed a definite increase throughout the infusion period. Following infusion of a fat emulsion there was a rapid rise in plasma fat to a value approximately twice the normal. As soon as the infusion was finished, however, the plasma fat began to decrease and in the short time of approximately one hour had returned to normal. Thus, apparently, infused fat leaves the blood stream rapidly.

Since the soybean phosphatide preparation used as an emulsifier had been found in previous work to be the best of a large number of emulsifying agents studied, it seemed worth while to attempt to fractionate the preparation with the object of removing the factors producing the granulomatous lesions. An assay method in albino rats was therefore devised (R. P. Geyer *et al.*, *J. Lab. Clin. Med.* **33,** *163 (1948)*) and by this means it was possible to find a chemical fraction of the soybean phosphatide which offers good emulsifying properties and little or no lesion-producing properties.

In studying the lesions produced by the emulsions it was noted that various substances were present in some of the giant cells. Some contained neutral fat while in others particulate matter was observed. In certain instances small bits of leather fragments were demonstrated, the latter probably coming from the leather gasket used in the homogenizer. By using plastic gaskets and passing the finished emulsion through a suitable filter, particulate fractions could be completely removed. The fraction which was finally evolved consisted mainly of phospholipids and represented most of those originally present.

In the pursuit of this study a photomicrographic technic was devised which allows accurate determination of the size of fat particles in emulsions (R. P. Geyer, G. V. Mann, and F. J. Stare, *J. Lab. Clin. Med.* **33,** *153 (1948)*). By means of high pressure homogenization, fat emulsions were prepared in which all particles were below 2 microns in diameter and most of which were beyond the resolving power of the light microscope. Factors conducive to the preparation of such emulsions were high pressure, high temperature, continuous recirculation of the material being homogenized, and the proper fat-stabilizer ratio.

The usual methods for determining blood lipids are laborious and time-consuming and even the microadaptations of these methods require quantities of blood large enough to prohibit repeated use in small laboratories. These workers consequently devised (*J. Lab. Clin. Med.* **33,** *175 (1948)*) a simple turbidimetric method for determining the level of infused fat in the blood after intravenous administration of a fat emulsion. Fat tolerance curves obtained by this means in the dog, rat, and rabbit indicate the rate at which infused fat leaves the blood stream.

CONVERSION OF TRYPTOPHAN TO NIACIN IN ANIMALS

The mechanism by which tryptophan substitutes for niacin in the diet of some animals and gives rise to increased excretion of niacin metabolites (*Nutrition Reviews* **5,** *247 (1947)*), continues to cause interest. P. Ellinger and M. M. Abdel Kader (*Nature* **160,** *675 (1947)*) cite evidence that the intestinal flora of animals is responsible for the major part of the conversion that takes place. These authors administered 100 mg. of DL-tryptophan to rats alternately by mouth and parenterally at three day intervals. The increase in urinary excretion of nicotinamide methochloride was two to five times greater after oral than after parenteral administration. This would indicate that the intestinal flora played an active role in the conversion. In other rats similarly fed, the urinary output of nicotinamide methochloride before and during the daily administration of 1 g. of succinylsulfathiazole was compared. When the Escherichia coli content of the feces was considerably reduced as a result of the medication, the test dose (100 mg.) of tryptophan was given. During medication, the nicotinamide methochloride excreted was reduced to from one-fifth to one-half that produced in animals not receiving the sulfonamide. Suppression of the bacterial flora by the sulfonamide thus apparently decreases the amount of niacin metabolites formed from tryptophan.

Since E. coli was thought to be the organism primarily responsible for the transformation, the effect of adding various amino acids to a synthetic medium on synthesis of nicotinamide by two fecal strains of this organism was determined. Under these conditions, synthesis of nicotinamide by these organisms was increased up to 450 per cent by ornithine, and 50 to 70 per cent by glutamine and arginine. Strangely enough, tryptophan did not increase production of nicotinamide by these cultures. When a mixed culture from the rat cecum was used, however, an increase of nicotinamide production was noted when tryptophan was added to the medium. These data would indicate that tryptophan is not the only amino acid which can stimulate bacterial production of niacin. Ornithine has been suggested previously on wholly *a priori* grounds as a possible precursor of niacin (M. Guggenheim, "Die Biogenen Amine," *S. Karger, Basel (1940)*); normally this amino acid is thought to be formed as an intermediate from dietary arginine, and is itself not a component of normal tissues so far investigated. The significance of this whole report, however, rests upon the magnitude of the observed niacin synthesis, and these figures are not given by the authors.

A short abstract by J. Schultz and G. T. Rudkin (*Federation Proceedings* **7,** *185 (1948)*) indicates that the ability to utilize tryptophan as a source of niacin is not common to all animals. In a chemically defined medium, and under bacteriologically sterile conditions, the fruit fly, Drosophila melanogaster, requires both tryptophan and niacin for growth. The requirement for niacin is enhanced by an increase in the tryptophan content of the medium, in contrast to the result obtained with higher animals. This observation may be interpreted to mean that the conversion of tryptophan to niacin is normally carried out by microorganisms of the tract, and when these are lacking, preformed niacin is required by these organisms. The observation may, on the other hand, reflect only a species difference. It is probable that some animal organisms may have retained ability to synthesize niacin from tryptophan, while others may have lost this ability.

These studies illustrate the difficulty in separating the biochemical activities of the intestinal flora from those of the host animal when studies of the intact animal are

made. Decisive experiments could presumably be made with the help of germ-free animals, as recently described in this journal (*Nutrition Reviews* **6,** *83* (*1948*)), and further results of Schultz and Rudkin, in which the niacin requirements of germ-free Drosophila are contrasted with those harboring microorganisms may prove illuminating.

PROTEIN AND ANTIBODY PRODUCTION

The hypothesis that depletion of dietary protein conditions inadequate response to infection was stimulated by epidemiologic studies during and after World War I (K. Faber, *Acta Tuberc. Scandinav.* **12,** *287* (*1938*); G. Schröder, *Beitr. Z. Klin. d. Tuberk.* **75,** *61* (*1930*)). This hypothesis has received considerable prominence based largely upon reports from P. R. Cannon's laboratory which have shown that severe protein depletion with associated hypoproteinemia leads to a marked decrease in humoral antibody elaboration by the rabbit and rat (*Nutrition Reviews* **1,** *186* (*1943*); **2,** *197* (*1944*); **3,** *78* (*1945*); **4,** *279* (*1946*); **5,** *106* (*1947*)).

In these studies antibody fabrication (antisheep erythrocyte and Eberthella typhosa vaccine) referred more to a technical procedure than to a mechanism of resistance beneficial to the host. Impairment of (gamma) globulin synthesis was largely inferred (P. R. Cannon, *Advances in Protein Chemistry* **2,** *135* (*1945*)). A further, somewhat more applicable test of the fundamental hypothesis has been reported from Cannon's laboratory by R. W. Wissler (*J. Infect. Dis.* **80,** *250*, *264* (*1947*)). These studies were designed to assess the relative abilities of protein deficient rabbits and rats to acquire immunity and subsequent resistance to bacterial infection. The data suggest that the depleted animals have distinctly greater mortality, somewhat lessened local phagocytosis, slightly altered leucocytic, and equivocally diminished humoral antibody responses to intradermal pneumococcal infection.

In the first series of experiments, five groups of rabbits, totaling 65 animals were used. There were 38 young (0.56 to 1.2 kg.) and 27 adult rabbits (1.8 to 2.5 kg.). In 27 of these, marked weight loss and protein depletion were achieved after ninety-six to one hundred and five days on a basal carrot diet plus a low protein cookie containing lard and corn starch supplemented with salts, brewers' yeast, and vitamins A and D. This diet afforded approximately a 2 per cent protein intake. Controls were fed at a level of 15 per cent dietary protein derived from a commercial rabbit food preparation supplemented by carrots biweekly. The deficient diet was not isocaloric with the control. It afforded about one-half the calories yielded by the control diet. It is also obvious that the nutritional difference between these two diets is more than a quantitative one in terms of protein. In one group of 5 rabbits, plasmapheresis facilitated protein depletion. After three to three and a half months on the respective diets, all animals with the exception of one rabbit in each group, were immunized by two intravenous injections of formolized, heat-killed pneumococcal vaccine. Six days after completion of immunization all animals were infected with 0.1 ml. of a young virulent culture of type I pneumococcus injected intradermally. Serum protein and hemoglobin concentrations were determined at time of vaccination. Agglutinin titers were determined six days after vaccination, just prior to infection. Leucocyte, and apparently chamber differential counts were obtained during the preinfection period and at five hour intervals during the twenty hour period following infection. Survival times were noted. Blood cultures in some, and tissue preparation for histologic study

in all nonsurviving animals were done at time of death.

The depleted animals showed marked weight loss or failure to gain, anemia, and reduced serum protein concentrations. The mortality data were striking; all nonimmunized rabbits irrespective of dietary status died. There was no difference in survival time between depleted animals and controls. In contrast to the susceptibility of the nonimmunized animals, 24 of the 27 depleted and 5 of the 28 control previously immunized rabbits died. Agglutinin titers, representing acquired immunity in response to vaccine, were less striking. In young animals comprising two of the five experimental groups, significantly lower titers were noted in the depleted rabbits. In the remaining three groups, the difference in titer between depleted animals and controls was less evident. In general, rather low titer levels were observed.

The data pertaining to total and differential white blood cell counts are difficult to interpret. In approximately half of the control rabbits an absolute increase in the number of white cells with essentially normal differential cellular distribution was observed after infection. A decrease in the white blood cells was noted in the remaining well fed immunized animals. In the deficient groups, relatively normal total white blood cell counts and differential counts were noted in the preinfection period. This was an interesting observation in view of the reported association of leukopenia and granulocytopenia accompanying protracted dietary protein deficiency (*Nutrition Reviews* **5,** *77* (*1947*)). Following infection, leukopenia with slight, relative granulocytopenia (or lymphocytosis) was noted in the immunized malnourished groups.

Hypoproteinemia, characterized by lowered serum protein concentrations and largely representing albumin depletion was observed in the animals on the deficient diet. Slightly greater concentration of sodium sulfate-precipitated globulin was noted in both diet groups following immunization. It was suggested that this increment partially reflected an increased quantity of antibody (presumably gamma) globulin. The largest fractionated globulin increment observed was 0.04 g. versus 0.7 g. in the deficient and control groups, respectively. Since gamma-globulin comprises approximately 10 per cent of the total globulin fraction, changes of the magnitude noted seem rather close to the limits of error of the salting-out method.

Depletion of albumin associated with hypoproteinosis appears to be correlated with a concomitant rise in alpha-globulin (B. F. Chow, *J. Clin. Invest.* **26,** *883* (*1947*)). Inference regarding serum antibody globulin postulated on the presented data, therefore, would be rather unreliable.

A further acute depression of serum protein concentration was noted in deficient animals in the immediate postinfection period. Although interpreted as acute protein loss, such apparent depletion might represent a hemodilution phenomenon. Acute pneumococcal infection may be associated with rather marked increases in plasma volume (H. C. Soule, T. E. Buckman, and D. C. Darrow, *J. Clin. Invest.* **5,** *229* (*1928*); D. D. Rutstein *et al.*, *Ibid.* **24,** *11* (*1945*)). The masking effects inherent in lability of the plasma compartment are not always fully appreciated (J. Metcoff and F. J. Stare, *New Engl. J. Med.* **236,** *26* (*1947*)). Acute nitrogen catabolism "toxic destruction" seems an unlikely etiology of the lowered serum protein, since protein catabolism subsequent to infection is thought to be less marked in deficient malnourished than in well nourished individuals (*Nutrition Reviews* **3,** *86* (*1945*)).

Evidence derived from pathologic review of the nonsurviving infected animals afforded little support for the initial hypothesis. Depleted animals evidenced varying degrees of organ atrophy and some fatty infiltration of the liver. Histopathology of the local lesion indicated that diminished

phagocytosis was evident in all nonsurviving animals, regardless of dietary status. Comparison of the local lesions of nonsurviving, immunized, depleted rabbits with those of the controls which had been killed indicated significantly more effective phagocytosis in the latter group. In view of the insignificant phagocytosis manifested by nonsurviving controls, it is possible that effective phagocytosis was an attribute of survivorship rather than of diet.

In a second paper (*J. Infect. Dis.* **80,** *264* (*1947*)), Wissler reports a repetition of these studies using 150 male adult albino rats. Seventy-six of these were depleted on a carrot-corn starch-lard diet—affording approximately 2 per cent protein and supplemented by liver, yeast, salts, and vitamins—continued for one hundred and thirty to one hundred and eighty-eight days. The control rats received a similar crude basal diet with substitution of 22 per cent casein for an equivalent amount of corn starch. The animals were grouped 4 to 7 per cage. In the four series of experiments reported, the general plan was similar to that carried out on the rabbits. As in the previous study, the reported data fail to indicate a significant difference between the post-immunization humoral antibody titers of depleted and control rats. In one experiment, the titers of depleted animals appeared to be slightly greater than those of the controls. This observation affords a sharp contrast to the previous work on sheep erythrocyte hemolysin in rats emanating from the same laboratory (*Nutrition Reviews* **3,** *86* (*1945*)). Perhaps the antibody fabrication mechanism initiated by a virulent pathogen may be somewhat different from that stimulated by a foreign protein antigen (*Ibid.* **4,** *279* (*1946*)).

As in the rabbit experiments, mortality data are striking. The depleted animals appear to have considerably less tendency to survive. The natural immunity of the rat to pneumococcus type I, titrated in terms of survival times of nonimmunized animals, was somewhat greater in the rat than in the rabbit. Depleted animals died in approximately half the required time for death of the controls. Diminished natural resistance secondary to protein deficiency was inferred. Mouse protection tests, however, using serums of both nonimmunized diet groups, failed to indicate depletion in protective circulating "natural" antibodies in the deficient rats. Injection of rabbit antipneumococcal serum prior to infection passively protected depleted, nonvaccinated, infected rats. Actively immunized depleted rats having slightly lower antibody titers died. In view of the differences of antibody titer (1 plus tube dilution), and the number of animals compared per group (4 to 5), the probability of the observation representing a chance occurrence seems rather great as suggested by the occurrence of 5 survivors in a group of 11 depleted animals having "acquired" immune titer levels similar to those observed in the group of 4 depleted "passively" immunized surviving rats. Some reservation, therefore, must attend the conclusion that passive immunization data in 4 rats—"supports the concept that the decreased resistance of the actively immunized protein-depleted rat is due in part to a lowering of the natural resistance and in part a decreased capacity to form specific antibodies."

White blood cell response to protracted malnutrition was not remarkable. Histologic review of the intradermal inoculation lesion suggested that well nourished immunized rats had more prompt active local phagocytosis than was exhibited by the depleted groups. This observation was compatible with the respective mortalities experienced, and was consistent with diminished phagocytic ability of granulocytes associated with dietary protein depletion reported by others (E. Gellhorn and J. O. Dunn, *J. Nutrition* **14,** *145* (*1937*); C. A. Mills and E. Cottingham, *J. Immunology* **47,** *503* (*1943*)).

On the basis of the observations it was

concluded that "from the practical standpoint the important fact is that protein-depletion in the rat appears to exert the double effect of diminishing both natural and acquired resistance to the pneumococcus."

The data presented in both papers corroborate the depressant effect of protracted malnutrition, including protein deficiency, on serum protein and hemoglobin formation. A considerable predisposition to survival was evidenced by the well nourished, in contrast to the deficient animals. The evidence does not provide a conclusive argument for the concept of impaired antibody response to infection in the protein depleted animal. Difference in both relative and actual survival subsequent to intradermal infection may be related to such things as local spreading factors and complement, in addition to local phagocytosis, humoral and tissue antibody production. Further variables such as host and pathogen response to infection in association with genetic characteristics and diet have been reviewed by H. A. Schneider (*Vitamins and Hormones* **4,** *35* (*1946*)).

Emphasizing the numerous, difficultly controlled, variables which prejudice the hypothesis supported by Cannon's group, an interesting study reported by M. M. Bieler, E. E. Ecker, and T. D. Spies (*J. Lab. Clin. Med.* **32,** *130* (*1947*)) further obscures the diet-protein, infection-response relation. The electrophoretic characteristics of the serum protein pattern of 29 patients, 8 having hypoproteinemia associated with nutritional anemia, were studied. Several immunologic attributes of serums from these patients, for example, complement titer, opsonic indexes, and antisheep hemagglutinin-titers were determined. The immunologic activity was correlated with observed electrophoretic serum protein variation. It was concluded ". . . that the sera of persons who are hypoproteinemic but have no infection can maintain certain immunologic functions even though a rather large proportion of the total serum protein has disappeared." And also that ". . . many malnourished patients with anemia . . . appear to be as resistant to certain diseases as well-nourished individuals." Finally, ". . . that in persons where hypoproteinemia exists because of a lack of materials with which to manufacture serum proteins, the limited amounts of materials available contributes to the production of certain important immunologic elements."

On the basis of accumulating experience and careful assessment of currently available data, it would seem that dietary protein represents a minor contribution rather than a major determinant conditioning response to infectious agents.

ROLE OF AMINO ACIDS IN CANINE EPILEPSY

The symptoms of canine hysteria and the experiments which led to the recognition of agenized (nitrogen trichloride-bleached) wheat flour, or more specifically the gluten of such flour, as the causative agent of this disease have been described previously in this journal (*Nutrition Reviews* **3,** *176* (*1945*); **5,** *184* (*1947*)).

In a continuation of previous studies of this condition, G. W. Newell, T. C. Erickson, W. E. Gilson, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **65,** *115* (*1947*)) observe that the convulsive seizures frequently seen during the running fits to which dogs are subject suggest that the condition might be related to epilepsy. Since typical changes in the electroencephalogram occur during epileptic seizures in man, a method for obtaining electroencephalograms in dogs was developed and applied to control and diseased animals.

Abnormalities in the electroencephalo-

gram (EEG) were observed in some animals as early as three days after placing the dogs on a wheat gluten ration; the maximum time required for any of 18 dogs to develop such changes was twenty-four days. Abnormalities in the EEG appeared before any clinical abnormality was noted. EEG records taken immediately after a generalized tonic clonic convulsion showed a typical seizure wave pattern; both this pattern and the interval EEG taken two days later were comparable to the corresponding patterns obtained in human epilepsy. No abnormalities were seen in the EEG patterns of control animals.

The marked similarity of clinical symptoms and electroencephalographic records of such diseased dogs to those of human epileptics was confirmed by M. L. Silver, S. S. Zevin, R. M. Kark, and and R. E. Johnson (*Proc. Soc. Exp. Biol. Med.* **66,** *408 (1947)*), who suggest that the disease in dogs be called canine epilepsy. These authors bleached wheat flour and fat-free wheat flour with nitrogen trichloride, and showed that the gluten fraction of the bleached fat-free flour showed the convulsive activity. Commercially prepared gluten has the lipid so firmly bound that it is not fully extractable. The present work excludes this lipid fraction of the gluten as the possible toxic ingredient resulting from agene treatment.

Although the agenized flour did not produce convulsions in monkeys, they did develop a tremor of the extremities and weakness of the hind limbs after five days on the experimental diet. The changes were not observed on the control ration. Inasmuch as previous workers (*Nutrition Reviews* **5,** *184 (1947)*) were unable to produce symptoms with agenized flour in several animal species other than the dog, including the monkey, the latter observations, which are not accompanied by experimental data or details, will require confirmation.

Since the protein fraction of wheat contains the toxic agent, M. L. Silver, E. P. Monahan, and J. R. Klein (*Proc. Soc. Exp. Biol. Med.* **66,** *410 (1947)*) prepared a mixture of amino acids to simulate the composition of gliadin. One half of this was kept as a control and the other half was "bleached" in the same manner as flour. The control and agenized mixtures were then administered intravenously via the femoral vein in dogs prepared for this procedure and for artificial respiration, and maintained in a paralyzed condition with dihydro-beta-erythroidine hydrobromide. The dogs used had received for two weeks previously a diet containing agenized flour. They showed abnormal electroencephalograms but no seizures during a preinjection period of one to two hours. Seizures followed the intravenous administration of the agenized amino acid mixture (0.2 to 1.5 g.) in all of 8 dogs tested, in less than five minutes. The untreated mixture of amino acids under the same conditions was ineffective in 4 dogs tested even when as much as 20 g. were injected over one hundred and twenty minutes.

In separate trials, dogs maintained on a control diet containing unbleached flour were similarly treated. The agenized amino acids again induced seizures in several dogs, definitely abnormal encephalograms in others, and no changes in a few. The authors believe that more uniform results were obtained in the dogs fed agenized flour because these animals had accumulated sufficient quantity of the toxic material via the alimentary tract to make them highly susceptible to an additional increment received via the blood stream.

It had been previously postulated that the toxic effect of the agenized flour was due to modification of certain amino acids present in the gluten, probably by entrance of chlorine into the tyrosine or tryptophan residues. In preliminary results with single amino acids, not reported in detail, these authors report that agenized arginine, glutamic acid, glycine, histidine, lysine, methio-

nine, tryptophan, and tyrosine are ineffective in inducing seizures. Results with proline, serine, and aspartic acid were equivocal, while agenized cysteine hydrochloride produced the seizures. No postulations as to the mechanism of the effect are given.

These results are the first to indicate that canine hysteria may result from chemical modification of a single amino acid. The authors point out that chemically modified peptides, derived from the treated gluten, might be more effective than the amino acids.

Once the modified amino acid or acids effective in producing the abnormal electroencephalographic patterns under these conditions are identified, it will be interesting to determine whether their administration with the diet will reproduce the entire syndrome, and if so, whether the syndrome results from competition between a modified amino acid and its parent structure, or by another mechanism.

IS THERE AN ASSOCIATION BETWEEN SOIL FERTILITY AND TOOTH DECAY?

E. Taylor (*J. Am. Dental Assn.* **29,** *438 (1942)*) observed a low incidence of dental caries among the native-born, continuous residents of Deaf Smith County, Texas, which he attributed partially to the presence of naturally occurring fluorides in the public water supplies at levels varying from 2.2 to 2.7 parts per million. In addition, he presented a brief series of values representing the calcium content of the soil and the calcium and phosphorus concentration for certain foodstuffs grown on that soil. According to his values, the soil had a high amount of assimilable calcium and the locally grown produce appeared to have higher phosphorus concentrations than that of other districts, and in some cases, particularly in flour, a higher calcium content. He believed that the high values for calcium and phosphorus in foods contributed to the low incidence of tooth decay and that water-borne fluorides could not be considered as the sole reason for the low susceptibility to carious lesions observed in this district. However, F. J. McClure (*Ibid.* **31,** *1091 (1944)*) presented much more detailed analytical data which indicated that the levels of calcium and phosphorus given by Taylor for foods grown in Deaf Smith County were not necessarily higher than comparable values for similar foods grown in other parts of the United States.

W. A. Albrecht (*Ann. Dentistry* **6,** *199 (1947)*) has made a survey of present knowledge of weathering factors affecting soil fertility, its effect upon the composition of foods, and the possible relation of varying degrees of soil fertility to the dental health of the residents in these districts. He draws attention to four facts that influence soil fertility and influence the variations in the quality of the crops produced by soils of varying fertility. First, there is an increase in annual rainfall from zero to about 60 inches in going from the Coastal Range of the Rocky Mountains eastward to the Atlantic Coast. The lower increases in rainfall give increased weathering of the rocks and thus increased construction of the soils. At about the mid-continental area with still greater rainfall, there begins to be an increasingly excessive soil development which results in an increasing soil destruction, both in the quantity and the quality of the soil. Second, where there is maximum soil construction in the mid-continental areas, there is a high ratio of exchangeable calcium to the exchangeable potassium on the colloidal clay of the soil, and a similar ratio of these two elements is reported to result in the crops grown thereon. Third,

calcium has been demonstrated to be vitally associated with the synthesis of proteins by plants, while potassium is associated primarily with the synthesis of carbohydrates. Fourth, as a result of items two and three, in soils under construction by limited weathering conditions, i.e., those soils with a higher calcium:potassium ratio, proteinaceous and mineral-rich crops and foods as well as high carbohydrate crops are commercially feasible, but in soils where excessive climatic forces have produced soils with low calcium:potassium ratios, the carbohydrate-producing plants grow best and are therefore cultivated most extensively, with the ultimate result that production of foods with high protein and mineral concentration is reduced while carbohydrate formation is increased.

In an examination of the soils of the United States and the crops best suited to these soils, Albrecht drew attention to the prairie soils where there is a high mineral and particularly a high calcium content as a result of the sparse rainfall and high evaporation. On these soils the protein-rich, mineral-providing forages flourish profusely and provide excellent grazing for beef, cattle, and sheep. By contrast, in the eastern half of the United States, the high rainfall has resulted in a high degree of leaching of the soils and a low calcium content. In primitive times, these soils supported the carbonaceous types of vegetation in the form of forests. When cleared they were best fitted for growing starchy crops by reason of their low calcium: potassium ratio and low mineral content, and only became capable of profitably producing high protein plants when adequately fertilized.

In an experiment described by Albrecht and performed by E. L. Graham of the Missouri Agriculture Experiment Station, the effect of the ratio of calcium to potassium in the soil on the protein content of soybeans was tested. Where the ratio was high, representing a soil under construction, the vegetative yield of soybean plants was actually one-fourth less than where the ratio of calcium to potassium was low. But this increased vegetative mass was accompanied by a reduction in the protein concentration by one-fourth, a reduction in the phosphorus concentration of one-half, and a reduction in the concentration of calcium by two thirds of that in the smaller weight yield of soybeans from the soil with high calcium: potassium ratio. The effect of such forage on the development of animal products was demonstrated in an experiment in which one group of sheep was fed lespedeza grown on soil fertilized by phosphate only, while another group was fed lespedeza grown on soil fertilized with both lime and phosphate. The wool of the first group was very sparse, coarse, and so heavily matted that it could not be carded, while the wool from the second group was normal and could be carded readily.

Further examples of the shift to lower protein crops was described in terms of wheat and corn. Districts where "hard" wheat with its higher protein content formerly was grown, now produce chiefly "soft" wheat with its higher acreage yields of low protein grain. In the case of corn, the volume has been increased per acre by hybrid vigor, while the concentration of protein in corn grain was stated to have decreased concomitantly from about 9.5 to 8.5 per cent during the last decade. These changes are largely unknown to the producer and the consumer because the urge, and also the demand, to produce greater quantities per acre have largely submerged any desire to improve quality if it should be at the expense of quantity.

The data presented by C. A. Schlack and J. E. Birren (*Science* **104,** *259 (1946)*) on susceptibility to dental caries of 69,584 naval inductees were segregated by Albrecht according to the districts of major degrees of soil fertility. These men represented 93 per cent of the original group from which the remainder had been eliminated for dental reasons. This selection may have

reduced the regional differences which Albrecht deduced. The United States was divided from east to west into five districts on the basis of soil fertility: the east coast; the central eastern states; the midwestern states; the far western states; and the west coast. The numbers of carious lesions per man for these districts were 17.55, 14.95, 12.08, 13.10, and 15.50, respectively. The east coast was further subdivided with the men from New England states having an average number of carious lesions of 21.3 in contrast to 19.6 for the men from the middle Atlantic states, and 13.4 for the men of the southern Atlantic states.

Further statistics on the general health were derived from a survey by L. M. Hepple of the University of Missouri who observed a higher number of draft rejections per thousand men examined in Missouri than in Kansas, which Albrecht felt was a reflection of the calcareous soils of Kansas in contrast to the lime deficient soils of Missouri. In a more detailed examination of the draft rejections within the state of Missouri on a regional basis, Hepple observed that in traversing the five major regions of the state of Missouri from the legume and cattle-raising area of the northwest to the cotton-producing areas of the southeast, the number of draft rejectees per thousand selectees increased progressively from 208, 247, 280, 339, to 368.

There are possibly other conditions which may have been superimposed upon the dental caries distribution described by Albrecht based on Schlack and Birren's survey of naval inductees. In a further study of the association between soil fertility and tooth decay, one would be interested in considering further the association between increased hours of sunshine annually and reduced incidence of tooth decay described by C. A. Mills (*J. Dental Research* **16,** *417 (1937)*) and by B. R. East (*Am. J. Pub. Health* **29,** *777 (1939)*). The relation of vitamin D to the incidence of dental caries has been reviewed previously (*Nutrition Reviews* **5,** *68 (1947)*). In addition, one should consider the distribution of fluorine and hardness of drinking water supplies in relation to caries susceptibility (*Ibid.* **5,** *322 (1947)*). Any one or all three of these variants may have had a causal relation to the data on regional caries susceptibility summarized by Albrecht.

The possible association described by Albrecht is one that merits a great deal of further examination to determine, not only for dental caries but for numerous degenerative diseases, whether the examples of lower food quality which have been related experimentally to soil fertility do have a causative relation to the differential incidence of these diseases in various parts of the country. In an age when there is so much interchange of food products among the various parts of any nation, one would expect that it would be difficult, in view of the present inadequate knowledge of the subject, to generalize very widely as to the relation of soil fertility to degenerative diseases. For example, in the heavily populated urban populations of the eastern seaboard, a large percentage of the cereal grains, beef, and lamb must come from the more fertile regions of the middle west. During winter months especially, appreciable quantities of the vegetables and fruits must come from the south and west. If a causative relation between soil and fertility and tooth decay should be demonstrated unequivocally then it would seem that the foods brought into the eastern regions might have reduced the susceptibility to carious lesions and possibly to degenerative diseases also, beyond what it might otherwise have been if all foods consumed had been locally produced.

As an immediate project to broaden the data on which Albrecht's association is founded, the examination of the individual determinations which were averaged to obtain the values in the "Tables of Food Composition in Terms of Eleven Nutrients" (*U. S. D. A. Misc. Pub.* **572** *(1945)*) might yield valuable information. Since these

data were collected by widely separated state agricultural experiment stations and from federal, commercial, and other laboratories, it would be possible to segregate them in relation to the district in which the original foods were produced. In this way, an already available pool of knowledge collected for another purpose might either strengthen or refute the premise that there is a wide and significant variation in food composition in relation to the soil fertility on which they were produced.

MICROBIOLOGIC VITAMIN ASSAY METHODS

The use of microbiologic assays for vitamins has gained almost universal acceptance during the decade since the development of yeast assay methods by R. J. Williams and co-workers and the first riboflavin assay by E. E. Snell and F. M. Strong using Lactobacillus casei. These experiments, which have become classic in this field, have been followed by many others which have improved and extended the various quantitative methods for routine use. Previous reviews (*Nutrition Reviews* **3,** *327 (1945);* **5,** *57 (1947)*) have cited a number of these articles.

During the past year new publications have appeared dealing with the determination of riboflavin, niacin, biotin, and folic acid. The total number of these papers is smaller than heretofore, perhaps due to general satisfaction with previously existing methods, as well as the fact that no new water soluble vitamins were characterized during this time.

A method for the determination of niacin which responds to as little as 0.005 microgram of the vitamin per 10 ml. of culture has been reported by N. Grossowicz and E. Sherstinsky (*J. Biol. Chem.* **167,** *101 (1947)*). The organism used is Proteus HX 19. The original medium, containing glucose, ammonium sulfate, and other salts was improved by the present authors to include hydrolyzed vitamin-free casein and sodium nitrate. After inoculation with the test organisms, the tubes are incubated in a slanting position at an angle of 10 to 15 degrees for forty hours at 30°C. Turbidimetric response to the vitamin is quantitative over a working range of 0.005 to 0.1 microgram per culture. Niacin and the amide were found equally effective for the organism, and ethyl nicotinate, nicotinylglycine, and the mono-and diethyl amides of niacin were active. Picolinic acid, quinolinic acid, and trigonelline were inactive. This organism is thus similar to a number of the lactic acid bacteria in its behavior toward various niacin derivatives.

The principal attractive feature of the Proteus assay appears to be its relatively greater sensitivity toward niacin; Lactobacillus arabinosus, the most widely used organism, is only about 10 per cent as sensitive. However, the fact that the organism does not produce acid may likely retard widespread adoption of the method. The greater sensitivity afforded by this assay would be of value chiefly with milk, eggs, certain fruits, and other materials of low niacin content.

An article by H. A. Kornberg, R. S. Langdon, and V. H. Cheldelin (*Ind. Eng. Chem. (Anal. Ed.)* **20,** *81 (1948)*) recommends the use of Leuconostoc mesenteroides 10,100 for the assay of riboflavin. This organism responds to as little as 0.001 microgram of riboflavin per 10 ml. of culture, and is thus twenty to fifty times as sensitive as the more widely used L. casei. This factor is of practical importance, since the riboflavin content of most natural materials is fairly low.

The growth medium used by these workers is a slight modification of the Snell-Strong formula, containing glucose, sodium acetate, boiled alkali-treated peptone, asparagine, cystine, salts, and a riboflavin-free yeast sup-

plement. The peptone in the present medium is boiled momentarily with alkali and then allowed to stand twenty hours at 37°C. to remove the last traces of riboflavin. The authors also mention that a more nearly synthetic medium may be used which includes hydrolyzed vitamin-free casein, glucose, several amino acids, purines, pyrimidines, vitamins, and salts. Inoculation is made with one drop of a washed heavy suspension of cells, and incubation of the assay tubes is carried out for sixteen or seventy-two hours, depending upon whether the determination is to be made turbidimetrically or by titration. Turbidimetric readings are made with a photocolorimeter using a 5400Å filter, and acid production is measured using 0.05 normal sodium hydroxide with bromthymol blue indicator. Using either technic, the authors report good agreement of assay values among the various levels tested, with recoveries of the added vitamin ranging from 90 to 100 per cent. Comparative assays of foodstuffs with L. mesenteroides and L. casei showed good agreement except with starchy substances of low riboflavin content, where the L. casei values were considerably higher. Since the extraction procedure involved only acid hydrolysis followed by neutralization and filtering, samples of white flour were extracted with ether and reassayed with both organisms. The L. casei values were thereby reduced to approximately the levels obtained with L. mesenteroides. The latter organism was reported to be stimulated by starch and fatty acids similarly to L. casei, but it was considered that the greater dilution of the samples which was possible in the L. mesenteroides assay reduced the stimulatory effect by these substances. Although the number of samples tested was not sufficient to establish the lack of influence of extraneous stimulatory materials, it appears from the data presented that ether extraction of cereals prior to riboflavin assay may not be necessary with L. mesenteroides.

Ether extraction to remove fatty materials appears to remain an important operation in the assay of biotin. In 1942, the stimulatory effect of lecithin and oleic acid upon L. casei was noted by J. C. Bauernfeind, A. L. Sotier, and C. S. Boruff (*Ind. Eng. Chem. (Anal. Ed.)* **14,** *666 (1942)*). That this effect is directly related to biotin metabolism for these organisms was shown by W. L. Williams, H. P. Broquist, and E. E. Snell (*J. Biol. Chem.* **170,** *619 (1947)*). Using L. arabinosus, L. casei, L. delbruckii, S. faecalis, and L. mesenteroides, these workers observed that 300 micrograms of oleic acid or 10 mg. of Tween 80 (a polyhydroxyethylene derivative of sorbitan esterified with oleic acid) were able to substitute for biotin in a medium containing hydrolyzed casein. They concluded that in the presence of the Tween and aspartic acid, biotin is not required at all by these organisms. Fatty materials in plasma which possess biotin-like activity (W. Trager, *Proc. Soc. Exp. Biol. Med.* **64,** *129 (1947)*) have been shown by K. Hofmann, A. E. Axelrod, and M. Mitz (*American Chemical Society Abstracts, 113th meeting, 42C (1948)*) to be entirely duplicated by a mixture of oleic, linoleic, and arachidonic acids. The replacement of biotin by these unsaturated acids would appear to make the above mentioned organism unsuitable for assay of this vitamin unless ether extraction is employed.

K. Hofmann, T. Winnick, and A. E. Axelrod (*J. Biol. Chem.* **169,** *191 (1947)*) have provided a means for a differential assay of biotin and oxybiotin. They observed that whereas biotin in alkaline solution could be quantitatively inactivated by reduction with Raney's nickel catalyst, oxybiotin was unaffected by this treatment and could be assayed with L. arabinosus in the usual manner. These relations held for biotin both in pure solution and in acid hydrolyzed yeast extracts.

Evidence has been presented which indicates that current procedures for extraction

of pantothenic acid may be inadequate (*Nutrition Reviews* **5,** *291* (*1947*)), since much of the vitamin occurs in bound form, including coenzyme A. The coenzyme is relatively unaffected by takadiastase and papain digestion, and it has been considered likely that autolysis during digestion is chiefly responsible for liberation of pantothenic acid. This view is supported by the work of J. B. Neilands and F. M. Strong (*Federation Proceedings* **7,** *176* (*1948*)), in which they report that an alkaline intestinal phosphatase and a pigeon liver enzyme together liberated about three times as much pantothenic acid from materials as was obtained after Mylase P digestion. In boiled chicken liver the new enzyme treatment yielded thirty-five times as much as Mylase P. No figures were given, but it was concluded that a large portion of the pantothenic acid present in natural materials has not been included in previous assay values.

CORNEAL AND LENS ABNORMALITIES IN AMINO ACID DEFICIENCY

Corneal vascularization similar to that observed in riboflavin deficiency and which also occurs in rats fed lysine or tryptophan deficient diets has been reported previously (*Nutrition Reviews* **1,** *327* (*1943*)). Likewise, deficiencies in histidine, leucine, methionine, and simple protein deficiency have also been found to produce corneal changes (M. E. Maun, W. M. Cahill, and R. M. Davis, *Arch. Path.* **40,** *173* (*1945*); **41,** *25* (*1946*); W. K. Hall, V. P. Sydenstricker, C. W. Hock, and L. L. Bowles, *J. Nutrition* **32,** *509* (*1946*)). The suggestion has been made (Sydenstricker, Hall, Hock, and E. R. Pund, *Science* **103,** *194* (*1946*)) that corneal vascularization may be a reaction resulting from a deficiency of any of the indispensable amino acids.

Sydenstricker, H. L. Schmidt, and Hall (*Proc. Soc. Exp. Biol. Med.* **64,** *59* (*1947*)) report on their studies to test this hypothesis. A synthetic diet composed of sucrose, salts, vitamins, and an amino acid mixture was fed to 25 day old rats. The amino acid mixture contained all of the indispensable amino acids and was fed at a level which supplied about three times the amount required for normal growth. Amino acid deficient diets were prepared by omitting the appropriate amino acid from this diet. Biomicroscopic examinations of the eyes of the rats were made three or four times weekly.

Some degree of corneal vascularization (defined as the situation where there is actual growth of capillaries in the cornea beyond the limits of normal variation) occurred on every amino acid deficient diet. Of the 7 animals in each group on deficient diets the numbers which showed corneal vascularization were, respectively: phenylalanine 7, leucine 6, isoleucine 7, arginine 5, histidine 7, threonine 7 (of 8 rats), and valine 5. Vascularization was most prompt and extreme in phenylalanine deficient animals; leucine and isoleucine deficient animals showed eye conditions which were almost as severe. Successively lesser degrees of vascularization were observed in histidine, valine, threonine, and least in arginine deficiencies. Rats deficient in leucine, isoleucine, phenylalanine, histidine, or threonine, when returned to the control diet (containing all of the essential amino acids) showed prompt regression of the condition. Thus corneal vascularization seems to result from a deficiency of any of the indispensable amino acids. Four of 7 rats on histidine deficient diets eventually developed bilateral cataracts; these were not observed in rats on the other deficient diets. Thus corneal vascularization is a much more frequent and general response to deficiency than is cataract formation.

This suggested to A. J. Schaeffer and E. Geiger (*Proc. Soc. Exp. Biol. Med.* **66,** *309*

(*1947*)) that tryptophan and histidine, in the absence of which cataract is commonly observed, might play some specific role in lenticular metabolism in addition to that common to all essential amino acids, i.e., protein synthesis. This concept was tested by the delayed supplementation technic discussed previously (*Nutrition Reviews* **5,** *316* (*1947*)). Young rats were given alternately a tryptophan deficient ration containing all other nutritional essentials, and a ration supplying tryptophan as the sole amino acid. The diets were interchanged at twelve hour intervals with one group of animals and at two hour intervals with a second group. In the latter group, all food was withdrawn for one hour when the diets were changed. Ingestion of the ration containing tryptophan was adequate to supply dietary requirements of this amino acid. Previously it had been demonstrated (*loc. cit.*) that tryptophan administration under these conditions does not permit growth on the deficient diet, apparently because amino acids cannot be stored in the body, and hence all must be present simultaneously to permit protein synthesis. It was argued that if tryptophan played a specific role other than in protein synthesis, for this purpose it would be furnished by the above mode of administration.

On examination, lenticular involvement (cataract) and corneal vascularization were noted in 10 of the 12 experimental animals. The 2 animals with no sign of cataract had been on the experimental diet only six weeks; in other animals there were usually, but not always, signs of involvement by six weeks. As in previous experiments, the alternate administration of tryptophan and of tryptophan deficient rations did not permit growth.

These observations resemble closely those observed on tryptophan deficient rations in the complete absence of tryptophan supplementation. Like growth promotion, cataract prevention by this amino acid can be accomplished only when it is supplied simultaneously with other essential amino acids. The experiments thus give no support to the hypothesis that tryptophan plays a role other than in protein synthesis in lenticular metabolism; they do not, however, exclude such a possibility.

Further work is required to explain why cataract is not observed in deficiencies of the other essential amino acids. It is not too difficult, however, to imagine circumstances under which deficiency of different amino acids would result in different symptoms, even though protein synthesis were the only role involved. It is known, for example, that several different proteins occur in lens tissue, and tryptophan and histidine may be peculiarly important to maintenance of an individual one of these, in whose absence cataract develops. Other possibilities may occur to the reader.

VITAMIN B_{12} AND PERNICIOUS ANEMIA

Despite positive hematologic responses obtained from folic acid in some cases of pernicious anemia, it has become clear that the action of folic acid differs from that of liver extract in the treatment of this disease. Folic acid is not always effective, therapeutically active liver extracts do not necessarily contain significant amounts of free or conjugated folic acid, and furthermore the neurologic symptoms of pernicious anemia are either not affected or become more severe with folic acid therapy (for summaries of this work see *Nutrition Reviews* **5,** *115*, *208*, *274* (*1947*); **6,** *90* (*1948*)).

Of special importance in this field is the report of E. L. Rickes *et al.* (*Science* **107,** *396* (*1948*)) of the Merck Research Laboratories which announces the isolation from

liver extracts of a crystalline compound which in only microgram quantities has given positive hematologic responses in initial tests on patients with Addisonian pernicious anemia. In this first paper the general background of the work is presented, including brief summaries of the microbiologic assay for the factor and the clinical trials, these latter being given in greater detail in succeeding articles.

Work on the purification of the "antipernicious anemia" factor has been greatly retarded by lack of sufficient patients for testing, and no other suitable test organism had been developed. M. S. Shorb (*J. Biol. Chem.* **169,** *455 (1947)*) had, however, reported that Lactobacillus lactis Dorner (LLD factor) required a factor which was found in purified liver extracts, and in concentrations which paralleled the antipernicious anemia activity of the extracts. It was in fact suggested that the LLD factor and the therapeutically active principle in liver extract were identical. Shorb (*Science* **107,** *397 (1948)*) has now reported that the crystalline material isolated by Rickes and co-workers is extremely active in the microbiologic test method, and that comparison with a standard liver extract indicates that the factor is either wholly or partially responsible for the LLD activity of the extract. Only 1.3×10^{-5} micrograms of the crystalline factor per milliliter of medium is required for half-maximal growth, hence the factor is among the most potent of microbiologically active compounds.

The factor has been tentatively named vitamin B_{12} which connotes only a nutritional significance and does not extend names indicating biologic roles which are not yet adequately studied. Vitamin B_{12} has been isolated in only "minute amounts," as a red crystalline material. Refractive indexes of the compound were reported, but apparently the small amounts available have thus far restricted further details on its physical and chemical properties.

Clinical trials by R. West (*Science* **107,** *398 (1948)*) have shown positive hematologic responses in initial trials with 3 patients. In the first patient, 150 micrograms of vitamin B_{12} were administered intramuscularly as a single dose. The reticulocyte count rose from 0.5 per cent to 27.0 per cent in five days, and the red blood cell count from 1.5 to 3.4 ($\times 10^6$) in twenty-three days. The other 2 patients received 6 and 3 micrograms of vitamin B_{12} respectively, and showed good reticulocyte responses and positive red blood cell counts and hemoglobin rises in the fifteen day period for which values were reported. These cases will undoubtedly be followed further, and the new material will be evaluated in other clinics.

The important question as to whether vitamin B_{12} can account for the therapeutic activity of liver extract has been considered. Such a comparison was made on the basis of the LLD activity of liver extracts and the crystalline compound. It was calculated that 1 microgram of vitamin B_{12} is equivalent to approximately 1 U.S.P. injectable unit of liver extract and to 11,000 LLD units. Since 1 U.S.P. unit is that amount required daily for satisfactory clinical and hematologic response, the 3 and 6 microgram doses administered to 2 of the test patients indicate the equivalence of vitamin B_{12} and liver extract in hematologic response. Earlier work with impure amorphous concentrates of vitamin B_{12} showed that 4 patients who received 20,000 to 40,000 LLD units (2 to 4 micrograms of vitamin B_{12}) gave strongly positive hematologic responses, while 3 patients who received 10,000 LLD units have weak or negative responses. This activity is in contrast to the 20 to 50 mg. of folic acid which have given hematologic response in some cases of pernicious anemia.

It is of course too early to determine what significance vitamin B_{12} has in relation to liver extract and whether it can replace liver extract in the treatment of pernicious

anemia. Will, for example, this single factor take care of the neurologic as well as the hematologic manifestations of the disease?

PROTEIN NUTRITION IN SURGERY

On general principles it might be supposed that a well nourished individual would withstand the stress of trauma or of an operative procedure better than someone malnourished or depleted. This has been the clinical experience of surgeons for many years, and it is supported at least in part by certain data derived from studies in both man and in animals (*Nutrition Reviews* **5,** *165* (*1947*)). Unfortunately, many patients for whom operations are necessary are poorly nourished when they consult the surgeon who must, when it is possible, undertake the arduous task of refeeding.

J. D. Stewart, H. W. Hale, and S. M. Schaer (*J. Am. Med. Assn.* **136,** *1017* (*1948*)) outlined the methods they used in managing protein deficiency in surgical patients. The authors dismissed discussion of the problem of supplying calories and nutrients other than protein by pointing out that the requirements for nutritional maintenance must be met. With the present day emphasis on protein nutrition, the importance of other nutrients, and particularly of providing sufficient calories, has sometimes been overlooked. Indeed, when complete parenteral feeding is undertaken, the limiting factor is calories rather than protein which may be supplied relatively easily as one or another of the protein hydrolysates commercially available.

To furnish intravenous protein, the authors used the enzymatic digest of casein and pork pancreas ("Amigen") containing 50 g. of digest and 50 g. of glucose per liter. It is assumed that the 50 g. of digest furnished an equivalent amount of amino acids. Actually the nitrogen content of this mixture is 12 g. per 100 g., or 37.5 g. of protein per 50 g. Furthermore, all of the amino acids and peptides are not used by the body since some of the free amino acids and a considerable quantity of the peptides are excreted in the urine apparently unchanged (*Nutrition Reviews* **6,** *53* (*1948*)). The authors recommend that at least three hours be allowed for the injection of 1 liter of the solution. Since 50 g. of protein approaches the minimum requirement it can hardly go far toward refeeding, and twice this volume would require more than six hours to administer. In addition to calories, the limitation of time in intravenous feeding is an important one.

The authors have given eight hundred and twenty-nine infusions to 166 patients with a reaction rate of 6.5 per cent. Sixty per cent of the reactions were pyrogenic in nature, 30 per cent due to nausea, and the remainder were allergic or associated with thrombosis of a vein. No contraindication was found to administration of this solution to patients with severe hepatic disease; in fact, good utilization was observed. This has been the experience of others (for example, J. H. Lewis, F. H. L. Taylor, and C. S. Davidson, *Am. J. Med. Sci.* **214,** *656* (*1947*)), but is contrary to the recommendation of the American Medical Association Council on Pharmacy and Chemistry (*J. Am. Med. Assn.* **136,** *692* (*1948*)), although no data or references are provided to support the opinion of the latter body.

In addition to the intravenous administration of nutrients, the authors discuss briefly intrajejunal feeding following jejunostomy. In comparison to the intravenous route, with jejunal feeding, as the authors point out, a mixture containing optimum quantities of all nutrients may be provided. The formula suggested provides in 2 liters nearly 200 g. of protein, almost entirely as casein. Although the caloric value is not

given, by calculation from the composition it is about 2200 calories per 2 liters. No fat is included, but may be added as desired, with the expected enhancement of the caloric value. Carbohydrate is supplied as sucrose. The mixture would appear to be a suitable one, although others have found combinations of common foods in liquid form to be satisfactory. Although halibut liver oil, vitamin K, and ascorbic acid are added to the mixture, the principal source of the vitamin B-complex is from 30 g. of yeast. This amount of yeast may not provide optimum quantities of the "B vitamins," especially of thiamine, unless it is fortified, or unless these vitamins are otherwise provided.

As the authors have made clear, the problem of feeding surgical patients who are ill is not a simple one, but requires patience and skill, as well as a good knowledge of nutrition.

COMPARATIVE ABSORPTION, EXCRETION, AND STORAGE OF OILY AND AQUEOUS PREPARATIONS OF VITAMIN A

Vitamin A, as one of the fat soluble vitamins, is normally present in the fatty portion of the diet. Partially for this reason, and partially because of its water insolubility, the rate at which it is absorbed is much slower than that which characterizes the water soluble vitamins. This rate appears to vary with the nature of the fat present in the diet, the nature of the compounds fed (for example, free alcohol, vitamin A esters) and a number of other factors, many of which are not clearly defined. The rate of absorption, as judged by blood levels following ingestion of test doses, is markedly decreased in various diseased conditions such as pancreatic fibrosis, celiac disease, and bile duct obstruction.

With the development of the great variety of dispersing agents now available, it has become possible to prepare aqueous dispersions of oils and fat soluble materials which behave in many respects as water solutions. The rate of absorption by children of vitamin A (as measured by increases in blood levels) from such aqueous dispersions of fish liver oil has been shown to be greatly increased over that observed with fish liver oil alone (*Nutrition Reviews* **5**, *286* (*1947*)). This was true both of normal children and of children showing the celiac syndrome.

In a continuation of these studies (A. E. Sobel *et al.*, *J. Nutrition* **35**, *225* (*1948*)) the question arose as to whether an increase in the blood level actually represents increased absorption of vitamin A. To settle this point, the liver storage test was applied, i.e., the actual amounts of vitamin A deposited in the livers of rats in response to ingestion of known amounts of vitamin A in aqueous or oily mediums were determined. Fifteen day old rats, together with their mothers, were placed on the U.S.P. XII vitamin A test diet. At 20 days of age the weanling rats were removed to experimental cages and continued on the vitamin A deficient diet ad libitum.

Vitamin A deficiency, as evidenced by cessation of growth or xerophthalmia followed in twenty-five to thirty days. Comparable groups of the deficient rats then received by stomach tube for three successive days 0.2 ml. of the various vitamin A-containing preparations. On the fifth day the rats were killed and the vitamin A content of each whole liver and the blood serum determined. Vitamin A concentrates from various sources were diluted in each case to the same degree in maize oil (the oily medium) or in water containing 16 per cent of Tween 20, a synthetic edible dispersing agent. A known dose, usually totaling between 1000 and 1400 U.S.P. units of

vitamin A, was given to each rat. When fed in oil, the mean liver deposit in rats fed vitamin A from three different sources was 131, 200, and 124 U.S.P. units. Equal unitage fed in aqueous dispersion resulted in deposition of 357, 378, and 400 U.S.P. units. The maximum deposition with aqueous dispersions was about 34 per cent of the dose fed. The marked differences found in the present investigation in absorption and deposition of vitamin A from oily and from aqueous mediums appear unequivocal. It thus seems highly probable that the sharp rise in the vitamin A tolerance curve for blood serum obtained with aqueous dispersions, as contrasted to the flat curves obtained with oily solutions, actually results from improved absorption and retention of vitamin A rather than merely representing a temporary high blood level.

This interpretation is confirmed by an independent investigation of G. R. Halpern, J. Biely, and F. Hardy (*Science* **106,** *40 (1947)*) who showed by bioassay with vitamin A deficient chicks, that an aqueous dispersion of the vitamin was far more effective than an oily solution in promoting growth.

Aqueous preparations of vitamin A have been shown to bring about higher blood levels of vitamin A in premature infants than did an oily product (S. Clifford, *Pediatric Research Soc., Skytop, Pa. (1946)*). Similar findings have been reported by J. M. Lewis, O. Bodansky, J. Birmingham, and S. Q. Cohlan (*J. Pediatrics* **31,** *496 (1947)*) in premature and full term infants, children, and adults. In 8 premature infants, the average rise of vitamin A in the blood five hours after 35,000 units of an oily preparation were administered was 62 units, after a similar dose of an aqueous preparation it was 274 units. The oily and aqueous preparations were given to 6 full term infants (35,000 units), 4 older children (5000 units per pound), and 6 adults (500,000 units) and vitamin A blood levels were determined after three, six, nine, and twenty-four hours. The average maximum rise in vitamin A concentration was 200, 600, and 1750 units in the respective groups when the oily preparation was administered; 1000, 4000, and 4500 units following the aqueous product.

The difference in blood levels of vitamin A produced by the two preparations was found to be due to a difference in intestinal absorption. Fecal excretion was determined in 7 infants who received doses of 12,500 or 35,000 units of each preparation. Stools were collected for twenty-four hours prior to, and for six days following the test dose. Vitamin A was determined by the method used previously in assaying the content of liver (J. M. Lewis, O. Bodansky, K. G. Falk, and G. McGuire, *J. Nutrition* **23,** *351 (1942)*). The fecal excretion varied from 11 to 60 per cent of the intake (average 38 per cent) following an oily preparation; from 2 to 17 per cent of the intake (average 7 per cent) when the aqueous preparation was given. The excretion was of the same order of magnitude regardless of dosage.

To investigate the storage of vitamin A in the body, 13,000 units were given in a single dose, in either the oily or aqueous vehicle, to 110 rats, while 39,000 units were given to 74 guinea pigs. Blood was obtained from the heart at three, six, nine and twenty-four hours, the animals being killed at the time of bleeding to determine vitamin A content of the liver. Excretion in the stools was measured in 12 rats and 12 guinea pigs. Blood levels of vitamin A were higher and fecal excretions lower in both animal species following administration of the aqueous as compared with the oily preparation. In rats, the vitamin A concentration in the liver rose from an average of 55 units per gram before therapy, to 1150 units in twenty-four hours after the aqueous preparation was given, and to 670 units following the oily product. The average amount of vitamin A stored in the whole liver was 58 per cent of the amount ingested in the aqueous medium, 30 per cent of that

ingested in the oily vehicle. In guinea pigs, liver storage amounted to 16 and 10 per cent of the intake of the aqueous and oily preparations respectively.

Smaller amounts of vitamin A, 1100 units per day, which is comparable to dosage in infancy, were given to 12 guinea pigs for two weeks. Higher blood concentration, lower fecal excretion, and greater storage of vitamin A were again demonstrated with the aqueous product.

One child who had cystic fibrosis of the pancreas and one adult with complete obstruction of the common bile duct were given each preparation of vitamin A. Marked increase in the concentration of vitamin A in the blood followed administration of the aqueous product while no rise occurred following the oily preparation.

The aqueous preparation contained vitamin A alcohol dispersed in an aqueous solution of propylene glycol containing a solubilizer, vitamin B components, and ascorbic acid, while the oily product contained vitamin A esters dispersed in fish liver oil. Less complete absorption of the esters might account for the lower concentration of vitamin A in the blood since S. W. Clausen, A. B. McCoord, and B. L. Goff (*Federation Proceedings* **5**, *129 (1946)*) have shown that vitamin A esters are hydrolyzed by lipolytic enzymes in the intestine, absorbed as the alcohol, and re-esterified in the intestinal wall. The authors stated, however, that data to be reported subsequently showed no difference in absorption of the alcohol, as compared with the ester, in either aqueous or oily mediums. Absorption was not influenced by either the vitamin B components or ascorbic acid which were present in the aqueous preparation. On the other hand, the amount of vitamin A absorbed was influenced both by the concentration of propylene glycol and by the solubilizer, mannitan monolaurate. Vitamin A distilled esters in an aqueous solution of Dextri-maltose were found to give rise to blood levels equal to those obtained when the alcohol was administered in aqueous propylene glycol.

Additional results bearing upon this problem are reported by C. D. May and C. U. Lowe (*J. Clin. Invest.* **27**, *226 (1948)*) who studied the vitamin A blood levels following the administration of unemulsified vitamin A esters, an emulsion of vitamin A acetate with Tween 20, unemulsified vitamin A esters plus pancreatin, an emulsion of vitamin A esters with Tween 20 and propylene glycol, crystalline vitamin A, and as controls Tween 20 and propylene glycol alone. Fourteen normal children, 5 with fibrosis of the pancreas, and 6 with steatorrhea associated with celiac disease were studied. No definite enhancement of vitamin A absorption from emulsification was observed in the normals. In contrast, the patients with steatorrhea and pancreatic fibrosis did show a distinct increase in absorption. Indeed, the children with pancreatic fibrosis had very poor or no absorption of the vitamin in the unemulsified form, but had virtually normal blood levels when the vitamin A acetate was administered. The two emulsifying agents alone produced no rise in the blood vitamin A in the normals but were not given to the children with pancreatic fibrosis or steatorrhea. The addition of pancreatin to the unemulsified preparation of vitamin A esters enhanced its absorption somewhat in the children with pancreatic fibrosis.

These studies demonstrate the importance of the vehicle in the absorption of vitamin A and suggest that investigation of the role of the vehicle in absorption of other fat soluble vitamins should be undertaken.

It has been stated that world supplies of vitamin A are insufficient to meet current needs. Results of this nature indicate that it may be possible through improved methods of administration to increase greatly the utility of present supplies. The exact reasons for the increased efficiency of utilization from aqueous dispersions are not known. Undoubtedly the small size of the individual vitamin A-containing particles is a major factor. It is possible, too, that the dispersing agents themselves, which are effective surface-tension depressants and wetting

agents, may play a role by modifying the intestinal surface in such a way as to promote absorption.

Reports in the literature on vitamin A requirements have been based on data obtained with oily preparations, both in animals (H. Goss and H. R. Guilbert, *J. Nutrition* **18,** *169* (*1939*); Guilbert, R. F. Miller, and E. H. Hughes, *Ibid.* **13,** *543* (*1937*)), and in man (L. E. Booher, E. C. Callison, and E. M. Hewston, *Ibid.* **17,** *317* (*1939*); J. M. Lewis and C. Haig, *J. Pediatrics* **15,** *812* (*1939*), E. L. Batchelder and J. C. Ebbs, *J. Nutrition* **27,** *295* (*1944*)). Little experimental evidence is available concerning the actual human requirement of vitamin A (*Nutrition Reviews* **4,** *67* (*1946*)). Further studies should include the use of aqueous as well as oily preparations, with determination of fecal excretion, to obtain a more accurate estimate of the vitamin A requirement of both animals and man.

STUDIES OF FISH TAPEWORM ANEMIA

An anemia which resembles pernicious anemia may be associated with an infestation with the fish tapeworm, Diphyllobothrium latum. This macrocytic anemia differs from true Addisonian pernicious anemia in a few important aspects—free gastric acid is frequently present and extensive involvement of the spinal cord is rare in the tapeworm anemia (I. W. Birkeland, *Medicine* **11,** *1* (*1932*)). This anemia responds to antipernicious anemia liver extract and stomach preparations. It is usually relieved by the expulsion of the worm without specific therapy if the diet provides sources of Castle's extrinsic factor (B. von Bonsdorff, *Acta med. Scandinav.* **105,** *516* (*1940*); **116,** *77* (*1943*)). These facts, coupled with the several demonstrations of the presence of intrinsic factor in the gastric secretions of patients with anemia caused by Diphyllobothrium, distinguish it in pathogenesis from true Addisonian pernicious anemia.

Studies which contribute to a further definition of the defect in tapeworm anemia have now been reported. It is to be recalled that W. B. Castle (*Am. J. Med. Sci.* **178,** *748* (*1929*)) demonstrated that beef muscle (source of extrinsic factor) was alone inactive in pernicious anemia, but when administered with normal human gastric juice (containing intrinsic factor) the combination promoted hematopoiesis. Yeast is also a source of the extrinsic factor. C. A. Hernberg (*Acta med. Scandinav.* (*Suppl.*) **123,** *255* (*1941*)) reported that gastric juice from patients with tapeworm anemia was active in "Castle's test" applied to true Addisonian pernicious anemia. B. von Bonsdorff (*Ibid.* **128,** *456* (*1947*)) now reports that a combination of extrinsic (meat or yeast) and intrinsic factor (normal human gastric juice) was inactive when fed to 7 patients with the tapeworm anemia, and possessed slight activity in 2 other such patients. The test was run, of course, prior to removal of the worm. The same preparations had good activity when tested in typical Addisonian pernicious anemia. Three of the patients with the tapeworm anemia who had failed to respond in Castle's test were subsequently treated with extrinsic factor after expulsion of the worm. They experienced good remissions. These findings are consistent with the hypothesis that Diphyllobothrium latum produces anemia by virtue of some destructive action on, or inhibition of, the interaction of the intrinsic and extrinsic factors. Furthermore, inasmuch as tapeworm anemia responds to the liver principle, these studies again point to a distinction between the action of the liver principle and the product of the interaction of Castle's extrinsic and intrinsic factors.

These observations were extended by von Bonsdorff (*Acta med. Scandinav.* **129,** *59* (*1947*)) in an effort to answer the questions: (a) Does the addition of Diphyllobothrium latum to a mixture of gastric juice and extrinsic factor destroy its efficacy in Addiso-

nian pernicious anemia? (b) Will the remission induced by removal of the worm be inhibited by feeding preparations of the tapeworm to the patient? (c) Is the antianemic property of hog stomach preparation decreased by the addition of Diphyllobothrium latum?

The Diphyllobothrium latum was obtained freshly expelled, frozen, ground, and suspended in water and added to the various preparations in amounts equivalent to some 30 to 150 g. of fresh worm daily. To answer question (a) the worm suspension was added to a mixture of intrinsic and extrinsic factors and then fed to 3 patients with Addisonian pernicious anemia. Good remissions were induced—hence, the addition of the worm did not destroy the antianemic efficacy of the extrinsic-intrinsic preparation. Question (b) was studied by treating 3 patients with anemia caused by Diphyllobothrium latum with a vermifuge, administering sources of the extrinsic factor, and observing the influence on the remission of the daily feeding of from 1 to 30 g. of Diphyllobothrium latum. The remissions "continued undisturbed" by the administration of the suspensions of the tapeworm. Finally, question (c) was investigated by observing the influence of the addition to a hog-stomach preparation of 15 g. of dried tapeworm per day. Two patients with Addisonian pernicious anemia were tested. Both subjects responded in a manner indicating that no compromise had occurred in the antianemic effect of the stomach preparation.

These latter studies indicate that the observed inhibiting effect of the tapeworm, Diphyllobothrium latum, on the antianemia activity of Castle's extrinsic-intrinsic preparation is restricted to a situation in which the worm is *in situ*. The effect is not evident when preparations of the worm are given to patients with Addisonian pernicious anemia or to patients experiencing a remission of anemia after removal of Diphyllobothrium latum. Furthermore, these investigations increase the evidence that the several macrocytic anemias are similar manifestations of a defective chain of processes in which there are several links.

METABOLISM OF ISOTOPIC NIACIN

N′-methylnicotinamide has been reported to be one of the principal excretory products of niacin in man and in several species of experimental animals (*Nutrition Reviews* **2,** *234* (*1944*); **3,** *263* (*1945*); **4,** *58* (*1946*); **5,** *110, 234* (*1947*)). However, niacin is not the only metabolite which is excreted as N′-methylnicotinamide, since the ingestion of tryptophan has been shown to result in a markedly increased excretion of this compound (*Ibid.* **5,** *247, 269* (*1947*)). At the present time it is not known whether other compounds similar to tryptophan and to niacin and its derivatives may likewise be excreted through this pathway.

J. M. Hundley and H. W. Bond (*J. Biol. Chem.* **173,** *513* (*1948*)) have studied the ability of the rat to convert niacin which had been labeled with C^{13} in the carboxyl position, into N′-methylnicotinamide. The labeled niacin was synthesized according to the method of A. Murray, W. W. Foreman, and W. Langham (*Science* **106,** *277* (*1947*)). The final product contained 0.81 per cent excess C^{13} which would correspond to an excess of 4.86 per cent of C^{13} in the carboxyl group. Eight male rats weighing an average of 339 g. had been maintained for several months on a niacin deficient diet on which rats excrete a negligible amount of N′-methylnicotinamide chloride. Then, the labeled niacin was fed in this diet at a level of 1 g. per kilogram of the above ration until 1 kg. of the diet had been consumed. Urine samples were collected daily under toluene for a fourteen day period. Estimation of the amount of N′-methylnicotinamide chloride excreted was made on the pooled sample by the fluorometric method of J. W. Huff and W. A. Perlzweig (*J. Biol. Chem.* **167,** *157*

(*1947*)). In all, 681 mg. of N′-methylnicotinamide chloride were excreted, which would be equivalent to 48.6 per cent recovery of the ingested, labeled niacin. The N′-methylnicotinamide chloride was extracted and recovered in crystalline form as the picrate and was in a sufficiently pure state that this crystalline material did not show any depression of the melting point when mixed with the picrate prepared from pure, synthetic N′-methylnicotinamide chloride. The free compound was then prepared from the picrate and crystallized from a minimum amount of boiling absolute ethanol. Examination of the free N′-methylnicotinamide chloride spectrophotometrically revealed a characteristic maximum at 2650 Å with no evidence of a maximum at 2600 Å, indicating that no nicotinamide was present. Fluorometric assays of the free N′-methylnicotinamide chloride and mixed melting point tests with a synthetic sample indicated the extracted material to be 100 per cent pure within the limits of weighing errors. When a representative sample of the N′-methylnicotinamide chloride was tested for C^{13} on the mass spectrometer, it was found that the concentration of isotopic carbon was almost as great as in the ingested, labeled niacin. When allowance was made for the fact that N′-methylnicotinamide chloride contains one more carbon atom than niacin, the tagged excretory product had 95.7 ± 0.7 per cent of the original C^{13} concentration representing a dilution of only 4.3 per cent.

Under the conditions of this experiment, therefore, almost all of the excreted N′-methylnicotinamide chloride arose directly from niacin, and only 4.3 per cent could have been formed from niacin and related compounds present in the carcass prior to ingestion of labeled niacin or from tryptophan or other sources during the feeding period. Since it was estimated that the carcasses of these rats contained sufficient unlabeled niacin and other related compounds prior to ingestion of labeled niacin to produce not over 6 per cent of the N′-methylnicotinamide chloride if entirely excreted during the experiment, little tryptophan would seem to have been used in the synthesis of this excretory product under the conditions of this experiment. However, the high amount of niacin (fifty times the normal requirement on this diet) which was made available to these rats may have been sufficient to inhibit the conversion of tryptophan to niacin. On the other hand, the conversion of tryptophan to niacin may only occur in appreciable amounts when the ingestion of niacin is low or when the ingestion of tryptophan is in excess of the bodily requirements. Under the circumstances of this experiment, the niacin available to the rats was in excess of normal, while the tryptophan content of the diet was about ample for growth and maintenance. A natural continuation of the present study would be one in which tryptophan is labeled in such a way that the amount of N′-methylnicotinamide chloride being formed from this source could be determined. One of the difficulties in these experiments is the necessity of isolating the N′-methylnicotinamide chloride from the urine in crystalline form. The detailed nature of this procedure probably makes it necessary to perform only a limited number of the desired combinations in any one experiment. The value of the data to be obtained from such a procedure is evident. Actually, the experiment of Hundley and Bond is the first absolute proof, other than a wide variety of circumstantial evidence, that niacin is converted to and excreted as N′-methylnicotinamide chloride.

MICROBIOLOGIC EVIDENCE OF THE IDENTITY OF ALPHA- AND BETA-BIOTIN

Two forms of biotin, differing in biologic activity and in structure, have been postulated to exist in natural materials. The first of these was isolated from egg yolk by F.

Kögl and B. Tönnis (*Z. f. physiol. Chem.* **242,** *43 (1936)*) and has been referred to in the literature as alpha-biotin. The second, beta-biotin, has been isolated from liver and milk by V. du Vigneaud, K. Hofmann, D. B. Melville, and P. György (*J. Biol. Chem.* **140,** *643 (1941)*; du Vigneaud, Hofmann, Melville, and J. R. Rachele, *Ibid.* **140,** *763 (1941)*; Melville, Hofmann, E. Hague, and du Vigneaud, *Ibid.* **142,** *615 (1942)*). The structure of the latter compound has been determined and it has been synthesized commercially for investigational purposes for several years now.

Although Kögl and W. A. J. Borg (*Z. f. physiol. Chem.* **281,** *65 (1944)*) have suggested a structural formula for alpha-biotin, no synthesis of this compound has been reported. The investigation in which Kögl and E. J. ten Ham (*Ibid.* **279,** *140 (1943)*) observed that beta-biotin had an activity approximately twice that of alpha-biotin for the growth of the "Rasse M" strain of yeast appears to be the major evidence around which the speculated difference in structure and biologic activity has developed. In addition, they presented some data on melting points, mixed melting points, and specific rotations of biotin and biotin methyl esters from egg yolk and liver which they interpreted as indicative that the two compounds with biotin activity were not identical. Investigators in this country were unable to demonstrate any difference by these tests for biotin samples prepared from liver, milk, and two other sources (D. B. Melville, *Vitamins and Hormones* **2,** *29 (1944)*). Du Vigneaud, Hofmann, Melville, and György (*loc. cit.*) observed that a sample of Kögl's biotin had an activity of only 40 per cent of their crystalline material (beta) for three microorganisms, yeast, Rhizobium trifolii, and Clostridium butylicum, but they felt that this difference should not be stressed in view of the possibility that the former sample might have deteriorated or been less pure than their preparation. The reported differences in biologic activity of these two materials would only be a valid conclusion if the two were of equal purity.

K. K. Krueger and W. H. Peterson (*J. Biol. Chem.* **173,** *497 (1948)*) have tested the comparative activity of a sample of Kögl's alpha-biotin and one of Merck synthetic d-biotin (beta). The sample of alpha-biotin had been prepared from egg yolk by Kögl prior to when the controversy of different activities had arisen and was said to contain 25 per cent impurity. Five organisms of diverse nature were used in these studies: two lactic acid bacteria, one anaerobe (Clostridium acetobutylicum), a yeast, and a mold. The comparative activity of Kögl's biotin and synthetic d-biotin for these test organisms was judged after growth under ideal conditions by the determination of the amount of acid produced by the two lactic acid bacteria, of the degree of turbidity resulting from the growth and development of cells in the yeast and Clostridium cultures, and by the weight of mycelium produced by the mold. In all test organisms, the activity of alpha-biotin when corrected for its 25 per cent impurity varied between 90 and 96 per cent of that of d-biotin assuming that all the l-biotin is inactive as demonstrated by J. L. Stokes and M. Gunness (*Ibid.* **157,** *121 (1945)*). On the basis of these data, Krueger and Peterson suggested two alternative assumptions: first, that the two compounds are identical; and second, that the two are chemically different but elicit the same microbiologic response. In view of the wide diversity of the microorganisms tested, the former assumption seems to have the greater strength. Some precedent for the latter assumption may be drawn from the fact that synthetic biotin and oxybiotin have equal activity for Lactobacillus arabinosus and L. pentosus 124-2. However, oxybiotin is much less active than synthetic biotin for yeast, Lactobacillus casei, and in the cure of egg white injury in rats (*Nutrition Reviews* **4,** *159 (1946)*).

NOTES

Letters to the Editor

Dear Sir:

In your excellent review of "Diet and Arteriosclerosis" (*Nutrition Reviews* **6,** *99* (*1948*)), it occurs to me that there is an important omission.

There is no reference to the work done by A. Steiner and F. E. Kendall (*Arch. Path.* **42,** *433* (*1946*)) on dogs in which they show that if feeding cholesterol and thiouracil is maintained over a sufficient period of time, the fatty plaques originally produced are eventually the site of irreversible changes, i.e., scar tissue and deposition of calcium which simulate human arteriosclerosis to a remarkable degree. The reason this reference is important is that it is a partial answer to the criticism of the earlier work in rabbits where irreversible changes did not occur.

Let me take this opportunity to add that I very much enjoy NUTRITION REVIEWS and find them a valuable addition to my reading habits.

J. Murray Steele
The Goldwater Memorial Hospital
Welfare Island
New York, N. Y.

Dear Sir:

In the recent review of the present knowledge of pteroylglutamates (*Nutrition Reviews* **6,** *131* (*1948*)) the possible relation of thymine (5-methyl uracil) to folic acid is discussed, the remarks being confined to the work of T. D. Spies and co-workers on human subjects.

It seems that the work of Spies *et al.* may be overemphasized as to its bearing on the folic acid problem in metabolism when one realizes that it has been demonstrated that thymine is not utilized by the rat in place of folic acid either for hematopoiesis (H. G. Petering and R. A. Delor, *Science* **105,** *547* (*1947*)) or for lactation (L. Mirone and L. R. Cerecedo, *Federation Proceedings* **7,** *294* (*1948*)), and that it does not replace folic acid for the chick (L. J. Daniel, M. L. Scott, L. C. Norris, and G. F. Heuser, *J. Biol. Chem.* **173,** *123* (*1948*)).

Whatever the effect of thymine is in pernicious anemia or sprue, it seems incorrect in the light of the animal work to designate its action as that of a vitamer of folic acid just as it seems incorrect to speak of folic acid as a substitute for the antipernicious anemia factor of liver.

H. G. Petering
The Upjohn Company
Kalamazoo, Michigan

Five Year Cumulative Index to Nutrition Reviews

The Five Year Cumulative Index to *Nutrition Reviews* is now in print. It covers the material which has appeared in *Nutrition Reviews* for the period November 1942 to December 1947. Many new entries which have not appeared in the annual indexes are included. The Index should be of considerable value to all who are interested in the science of nutrition. A limited supply is available at the Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. at $1.00 each.

Breast Feeding

A subcommittee of the Committee on Maternal and Child Feeding of the National Research Council has reported its recommendations concerning the advisability of breast feeding for infants (*J. Am. Med. Assn.* **135,** *915* (*1947*)).

The report is divided into three sections. In the first, breast milk as a food is discussed, and it is pointed out that under most circumstances breast milk contains "adequate quantities of food elements for good nutrition in the early months of life," although food and vitamin supplements

should be added after the first few weeks. The preventive and therapeutic nature of breast milk in certain gastrointestinal disturbances is discussed.

In the second section, the importance of breast feeding in the emotional development of the infant is discussed. The lack of scientific data on this important subject is emphasized, but with the evidence available it is suggested that breast feeding should be given the benefit of the doubt. The sense of security and gratification which may be provided to the infant (as well as to the mother) are considered by many to be of utmost importance in its future development.

The third section is concerned with the contraindications to breast feeding, such as maternal illness or diseases of the breast.

The report is a brief but mature discussion of an important problem, and the recommendations have the support of the Food and Nutrition Board of the National Research Council, as well as the Committee on Maternal and Child Feeding.

Similar conclusions are reached by S. S. Stevenson (*J. Pediatrics* **31,** *616 (1947)*), who in addition suggests that the lower vitamin A and ascorbic acid content of cow's milk are not made up by the present allowances of the Food and Nutrition Board of the National Research Council. He recommends that 220 I.U. of vitamin A per pound of body weight and 100 mg. of ascorbic acid be given daily when artificial feeding is necessary.

Oral and Intravenous Phenylalanine Requirement for Man

Little is known of the quantitative requirements in man for individual amino acids. Particularly lacking are data on the amount of each amino acid needed when the source of nitrogen is a protein hydrolysate administered intravenously. The subject is pertinent because of the widespread use of such hydrolysates for protein feeding. R. D. Eckhardt and C. S. Davidson (*J. Clin. Invest.* **27,** *165 (1948)*) present evidence that the phenylalanine requirement to maintain nitrogen equilibrium in man is greater when supplied in a protein hydrolysate given intravenously than when the hydrolysate is given by mouth.

For their investigations they used a complete acid hydrolysate of casein which was free of glutamic and aspartic acids and supplemented with tryptophan, methionine, and glycine. This amino acid solution was given either orally or intravenously as the sole source of nitrogen in the individuals studied, and contained from 1.7 to 1.9 g. of phenylalanine per liter. The amount of hydrolysate given daily (1 liter) furnished 14 to 15 g. of nitrogen. The protein-free basal diet provided about 3000 calories daily and was said to be adequate in vitamins and minerals. The total urinary alpha-amino nitrogen was measured by the gasometric ninhydrin method, and phenylalanine in the hydrolysate and in the urine by microbiologic methods.

These normal adult males were given the hydrolysate as a sole source of nitrogen. When given orally the amount of phenylalanine contained in the mixture was enough to maintain nitrogen equilibrium, but when given intravenously, negative balance ensued unless a supplement of phenylalanine was given (1.0 g. by mouth). When the hydrolysate was given by mouth, 1.7 per cent of the administered phenylalanine was excreted in the urine. The excretion was increased from 2.1 to 6.5 per cent when given intravenously together with the oral supplement, and further increased from 10.5 to 11.6 per cent during the periods of negative balance when the phenylalanine supplement was omitted. The authors point out that 1.9 g. of phenylalanine given orally may not be the minimum requirement, nor 2.7 g. the "intravenous minimum." However, 1.9 g. was not sufficient to maintain nitrogen equilibrium when the hydrolysate given intravenously formed the sole source of nitrogen.

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The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

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